TECHNICAL MEMORANDUM: TOPICS RELATED TO THE TRIBUTYLTIN STUDY AT THE HARBOR ISLAND SUPERFUND SITE, WATERWAY SEDIMENT OPERABLE UNIT

1.0 INTRODUCTION

The purpose of this technical memorandum is to address topics of interest identified by the U.S. Environmental Protection Agency (USEPA) and other agency reviewers on issues related to the findings presented in *Tributyltin in Marine Sediments and the Bioaccumulation of Tributyltin: Combined Data Report* (ESI 1999b), prepared for the West Waterway portion of the Harbor Island Superfund Site, Waterway Sediment Operable Unit (WSOU). Some agency reviewers indicated that the measured TBT bioaccumulation in test organisms from the West Waterway tributyltin (TBT) study was less than they would have expected from the sediment and porewater TBT concentrations measured in site samples. This concern was based in part on a comparison of the bioaccumulation test results with studies reported in the literature and with other similar studies performed in the general Harbor Island area. Reviewers suggested that several test parameters (e.g., species selection, exposure regime of tests, organism health) may have influenced the results. This memorandum discusses and responds to those concerns. This TBT study was performed in accordance with a Sampling and Analysis Plan (SAP; EVS 1998a) that was reviewed and commented on by all reviewers prior to approval of the SAP by USEPA, and resultant data from the TBT study were determined to be of high quality (ESI 1999b). Finally, this memorandum was prepared per USEPA request, and was not required by the Administrative Order on Consent signed May 14, 1998, by the property owners, the Port of Seattle, and USEPA that formally outlined the objectives of the original study.

TBT was identified as a contaminant of potential concern in the sediment of the WSOU during the remedial investigation sponsored by USEPA under Superfund (WESTON 1994). Currently, there are no Federal or State sediment quality guidelines or standards for evaluating TBT concentrations in sediment. An interagency work group was formed to identify and evaluate approaches to deriving an effects-based sediment cleanup concentration for use in Puget Sound (USEPA 1996). Initially, the work group completed a literature search and review of toxic effects associated with exposure to TBT in the marine environment. Most of the available literature presents toxicity of TBT for water, and only two studies (covering four species) evaluated toxicity associated with sediment concentrations of TBT (see Section 3.1, USEPA 1996). The work group also considered calculation of Apparent Effects Threshold (AET) values for TBT using

chemical (bulk sediment) and biological (sediment toxicity, benthic infauna) data collected in Puget Sound, Washington (see Section 2.3, USEPA 1996). However, the existing Puget Sound data did not support a clear identification of an AET value for TBT. A maximum no-effect concentration could often not be established because, in several cases, the highest sediment TBT concentration associated with no biological effects also was the highest concentration measured among all the stations sampled. Also, good correlations were not found between bulk TBT sediment concentrations and laboratory toxicity and *in situ* benthic community responses. Based on an evaluation of available information, the work group concluded that bulk sediment concentrations of TBT was a poor predictor of bioavailable TBT (USEPA 1996). Further, the work group recommended that when TBT is a contaminant of concern in sediment, porewater concentrations of TBT should be measured, and toxicity testing or bioaccumulation testing (*in situ* or laboratory) be conducted to confirm the ecological significance of concentrations measured in porewater. The work group did not provide any recommendations for specific bioaccumulation test species, because it was believed that additional work needed to be done to identify the most appropriate species.

Under an Administrative Order on Consent with the USEPA, a consortium of Harbor Island waterfront property owners evaluated the bioavailability and the potential effects associated with TBT in sediments in the WSOU. The overall purpose of the work was to develop a site-specific, effects-based tissue trigger concentration that could be used to determine the need for remediation of TBT-contaminated sediments in the WSOU. Effects considered relevant for the development of a site-specific trigger concentration included mortality, reduced growth, and reproductive impairment. Other commonly reported sublethal effects, such as bivalve shell thickening or induction of imposex or intersex in gastropods, were not included in the evaluation because these endpoints have no established connection to population-level effects, and because there is a lack of suitable habitat at the site for the typically affected species—oysters, mesogastropods, and neogastropods. The WSOU is a deep (-30 to -60 ft MLLW), industrialized waterway within the Duwamish River estuary. Very little intertidal habitat is available because of extensive channelization and dredging of the waterway, and no commercial or recreational shellfish beds occur. In addition, gastropods typically are not a large component of the Duwamish River estuary benthic community, and mesogastropods and neogastropods make up only a small part of the total gastropod abundance (ESI 1999a, Appendix D).

The evaluation of TBT in WSOU sediments was conducted in two studies. First, the literature was reviewed to identify paired tissue residue and effects data for marine invertebrates and fish (ESI 1999a). The tissue residue data were used to develop a site-specific, effects-based trigger concentration (ESI 1999a). Second, sediment samples were collected throughout the WSOU for chemical and biological testing (ESI 1999b).

TBT concentrations were measured in bulk sediments and porewater samples; a subset of sediment samples collected was used for bioaccumulation testing. Bioaccumulation testing was conducted to determine site-specific exposures to two marine invertebrate species—a bivalve (*Macoma nasuta*) and a polychaete (*Nephtys caecoides*). No approved marine sediment toxicity bioassay protocols for test species that have demonstrated a sensitivity to TBT were available (USEPA 1996), so no toxicity testing was conducted. The resulting tissue TBT concentrations were then compared to the effects-based trigger concentration derived from the literature (ESI 1999b).

Bioaccumulation testing was selected for this site because of the many advantages of using tissue concentration data. As stated in USEPA (1999), tissue concentration data integrate multiple exposure pathways (dermal contact, ingestion, respiration) and provide a direct measure of exposure that is only predicted or assumed under other methods. Tissue data also can address food chain effects that are not represented in other current assessment methods. When the aquatic organism is used as human food, tissue concentrations can provide a direct link between human health and sediment criteria (USEPA 1993). When combined with effects measures, tissue concentration data can strongly link a source of contamination with impacts.

Section 2.0 of this memorandum discusses the conditions of the bioaccumulation testing undertaken for the WSOU TBT study. Recent studies examining the bioaccumulation of TBT are discussed in Section 3.0. Section 4.0 presents the references cited.

2.0 BIOACCUMULATION TESTING FACTORS

2.1 Species Selection

N. caecoides and *M. nasuta* are benthic invertebrates that appear on the preferred list of test organisms identified by the local State and Federal agencies for testing bioaccumulation potential from marine sediments (DMMP 1998). Other national guidance documents that address sediment bioaccumulation testing also include these two genera in lists of recommended test organisms:

> ASTM E 1688: Standard Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates (ASTM 1997)
>
> USEPA Guidance Manual: Bedded Sediment Bioaccumulation Tests (USEPA 1993)
>
> USEPA and U.S. Army Corps of Engineers: Evaluation of Dredged Material Proposed for Ocean Disposal (Green Book) (USEPA/USACE 1991)

U.S. Army Corps of Engineers and USEPA: Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. (Inland Testing Manual) (USEPA/USACE 1998)

In the above-referenced documents, *M. nasuta* is a recommended benchmark species for bioaccumulation testing. Although *N. caecoides* is not identified in these documents as a "recommended" bioaccumulation test species, *Nephtys* sp. is identified as a "suitable" test species (USEPA/USACE 1991) and *Nephtys incisa* is identified as "secondary" test species (ASTM 1997) and a "potential" test species (USEPA 1993).

Critical in selecting species for testing sediment contaminant bioavailability is selecting species that ingest sediment (see ASTM E 1688). The feeding strategies of the bivalve *M. nasuta* have been extensively studied (USEPA 1993). *M. nasuta* is a facultative filter-feeder, meaning that the species will feed on surface sediments to obtain nutrition, provided other sources of food, such as phytoplankton and resuspended particles, are not available (USEPA 1993). Laboratory bioaccumulation tests are conducted so that resuspension of bedded sediment does not occur, and use filtered seawater so that plankton are not introduced into the exposure system; thus, it is anticipated that the clams will be feeding directly from the surface sediments during the test.

The feeding strategies of *Nephtys* spp. are less well studied, the most common citation being work published by Fauchald and Jumars (1979). Their work summarized the information known at that time concerning the feeding strategies of polychaetes. Fauchald and Jumars (1979) identified more than 103 nephtyids, which are all free-living burrowers that may periodically form poorly agglutinated burrows. Based on extensive studies, Fauchald and Jumars (1979) identified *Nephtys* spp. as carnivores. They also noted two studies on the East Coast that suggested that *N. incisa* may ingest sediment as a subsurface deposit feeder. In summarizing the feeding guilds for each polychaete family, Fauchald and Jumars (1979) identified the dominant guild for Nephtyidae as carnivores and a secondary guild of subsurface deposit feeders (Table XXXI, Fauchald and Jumars 1979).

A recent study was conducted with 11 polychaete species to determine if feeding modes were correlated with differences in the ratio of enzyme activity for oipase and protease and the surfactancy of gut fluids (Mayer et al. 1997). The authors concluded on the basis of these two factors that field-collected *Nephtys caecoides* was a carnivore.

In the West Waterway study, prey organisms were presumed not to be present as a food source because they were screened from the site sediments prior to the bioaccumulation test. Thus, sediment was presumed to be the only potential food source.

Another consideration in selecting test species was the fact that the organisms can coexist in the same aquaria resulting in reduced sediment testing volume requirements and setup costs (Kendall 1996). Battelle developed the protocol for concurrent testing of *M. nasuta* and *N. caecoides* and the protocol was accepted by the PSDDA agencies in 1996.

In summary, the exposure of *Macoma* sp. and *Nephtys* sp. to sediment contaminants may be determined, in large measure, by the test conditions (e.g., in the field *Nephtys* sp. is a predator, and in laboratory test chambers their feeding strategy is unclear), by the character of the sediment (e.g., sediment grain size, TOC content), and by the flow regime during the test (e.g., static renewal or flow-through). These conditions may affect the exposure pathways that result in tissue burdens in the test organisms. It is unknown whether or not these factors contributed to the low accumulations measured in the West Waterway laboratory bioaccumulation tests.

2.2 Testing Conditions

The testing protocol used for the bioaccumulation tests were derived from USEPA's national guidance manual on conducting studies with bedded sediment (USEPA 1993). The testing protocol used in this study was approved by USEPA in the SAP (EVS 1998a). Quality assurance and quality control (QA/QC) parameters were measured throughout the test, and the observations were within the associated acceptable range. Two important factors were taken into consideration when designing the tests: 1) adding supplemental sediment during the course of the exposure period, and 2) using low-volume seawater flow-through. As discussed by USEPA (1993), the rationale for introducing additional sediment to the test chambers during the exposure period is to provide fresh organic material for ingestion by the test species, to ensure continued exposure to the contaminants of concern. USEPA (1993) and ASTM (1997) recommend either flow-through or static (preferably static-renewal) exposure systems to ensure that the dissolved-oxygen concentration remains greater than 60 percent of saturation during the exposure period. USEPA (1993) states that although flow-through conditions are desirable, they are not normally required for a successful bedded sediment test.

Given that both species were tested in the same exposure chambers (Kendall 1996) and that the oxygen demands of the sediments to be tested were not known beforehand, a flow-through exposure system was selected. Flow-through rates provided for approximately four complete water exchanges per day, slightly less than the range recommended in the Inland Testing Manual for Dredged Material (e.g., water exchanges range from 5 to 10 volumes per day) (USEPA/USACE 1998), meaning that water retention times were greater than those required by national guidance.

2.3 Organism Health

Two indicators of organism health were measured at the end of the exposure period: organism survival rate and tissue lipid content. Lipid content is recommended by USEPA (1993) as a suitable indicator of organism health. Under non-stress conditions, invertebrates will acquire and store food reserves as lipid. Under conditions of poor nutrition, contaminant exposure, or other stresses, invertebrates will deplete their lipid reserves, then begin to catabolize protein (Capuzzo and Lancaster 1982; McKenney 1982; Johns et al. 1985). Given that the lipid concentrations in the tissue of both test species were similar to controls at the end of the exposure period (e.g., generally ranged from 3 to 5 percent dry weight for *M. nasuta*, and from 5 to 8 percent dry weight for *N. caecoides*), the test organisms were considered by the respondents to be in good physiological health during the exposure period. However, because lipid concentrations were not measured in test species at the initiation of the test, some believe that the health of the test organisms cannot be ascertained by only end-of-test lipid measurements. No other measures of organism health were identified as viable measures for the organisms being tested. For example, growth also is a measure of physiological health, but the adult age class for both species was used in the test, and adult polychaetes and bivalves typically do not grow significantly during adulthood, or they grow at such a slow rate that the measure is not sensitive. As a matter of practice, ASTM (1997) and USEPA (1993) recommend that bioaccumulation tests be conducted with organisms that grow very slowly.

3.0 CASE STUDIES

In this section, the results of the WSOU sediment bioaccumulation testing (ESI 1999b) are compared to the results of several field and laboratory bioaccumulation studies. The results are compared in terms of the reported bioaccumulation factors (BAFs) and biota to sediment accumulation factors (BSAFs). These ratios are based on the relationship between the sediment contaminant concentration and the tissue contaminant concentration. They are calculated using the following equations:

$$BAF = \frac{\text{tissue concentration}}{\text{sediment concentration}} \qquad \text{Equation 1}$$

$$BSAF = \frac{\text{lipid normalized tissue concentration}}{\text{organic carbon normalized sediment concentration}} \qquad \text{Equation 2}$$

The BAFs and BSAFs calculated for the WSOU tests as well as a range of field and laboratory bioaccumulation studies for TBT are presented in Table 3-1. The results of each study are discussed in the following sections. Although data also were available in Bryan et al. 1989, sediment data were collected one to two years prior to the collection of organism tissue, so BAFs were not calculated using those data.

3.1 WSOU Bioaccumulation Testing

Twenty sediment samples collected throughout the WSOU were submitted for bioaccumulation testing with *M. nasuta* and *N. caecoides* (ESI 1999b). The mean BAF for *M. nasuta* was 0.49; for *N. caecoides* it was 1.2 (Table 3-1). The mean BSAFs were 0.17 for *M. nasuta* and 0.24 for *N. caecoides*.

3.2 Coos Bay, Oregon

Five sediment samples were collected along with resident mussels and oysters from the Isthmus Slough in Coos Bay, Oregon (McCann pers. comm. 1999). The range of bulk sediment TBT concentrations was 174 to 930 μg TBT/kg dry wt. The calculated BAFs are presented in Table 3-1. The BAFs were less than the BAFs calculated for the WSOU sediments. The low tissue concentrations are consistent with the fact that there have been no reports of oyster shell thickening in this area.

3.3 T-18 Bioaccumulation Testing

Bioaccumulation testing was conducted in order to determine the suitability of sediment from the Port of Seattle Terminal 18 for open-water disposal (EVS 1998b). TBT was identified as a chemical of concern because sediment concentrations exceeded bioaccumulation triggers established by the Puget Sound Dredged Disposal Analysis (PSDDA) agencies. Sediment bioaccumulation testing was conducted as part of a PSDDA Tier II bioaccumulation evaluation. TBT was measured for three samples (Table 3-1). The BSAFs were calculated using mean lipid values from the WSOU study because lipid data from the T-18 study were not available.

The T-18 bioaccumulation testing was similar to the testing conducted for the WSOU. One important difference between the studies was that no renewal of sediment was done during the T-18 testing. The resulting BAFs were higher in the T-18 testing than in the WSOU testing for the same test species. For *M. nasuta*, the BAF calculated for T-18 was approximately ten times higher than the mean value obtained for the WSOU testing. For *N. caecoides*, the T-18 BAF was approximately four times the mean value for WSOU. Differences between these datasets are difficult to intrepret because of the differences in the number of samples. *M. nasuta* accumulated higher tissue concentrations than *N. caecoides* in the T-18 testing; the opposite relationship was observed in the WSOU bioaccumulation testing.

Table 3-1. Calculated BAFs and BSAFs

STUDY	EXPOSURE	ORGANISM	N	BAF	BSAF
Bryan and Gibbs 1991	Field exposure	Ragworm (*N. diversicolor*)	2	1.08-2.46	na
	Field exposure	Periwinkle (*L. saxatilis*)	1	0.34	na
	Field exposure	Periwinkle (*L. littorea*)	1	2.27	na
	Field exposure	Bivalve (*P. pholadiformis*)	1	1.88	na
	Field exposure	Clam (*S. plana*)	1	7.58	na
	Field exposure	Cockle (*C. edule*)	1	9.28	na
	Field exposure	Clam (*M. balthica*)	1	10.3	na
	Field exposure	Clam (*M. mercenaria*)	1	19.4	na
	Field exposure	Clam (*M. arenaria*)	1	82.7	na
ESI (1999b)	Laboratory exposure to field collected sediment	*M. nasuta*	20	0.49 ± 0.42	0.17 ± 0.1
		N. caecoides	20	1.2 ± 0.7	0.24 ± 0.1
EVS (1998b)	Laboratory exposure to field collected sediment	*M. nasuta*	3	5.22 ± 0.63	5.82[b]
		N. caecoides	3	2.12 ± 2.05	2.27[b]
King et al. (1989)	Field exposure	Oysters	8	2.97 - 597	na
Kure and Depledge (1994)	Field exposure	Periwinkle (*L. littorea*)	4	5.5 - 21	na
		Clam (*M. arenaria*)	2	127 - 204	na
Langston et al. (1987)	Field exposure	*N. diversicolor*	16	3.0 ± 0.6	na
		M. arenaria	8	77.9 ± 20.5	na
Langston and Burt (1991)	Laboratory exposure	*Scrobicularia plana*	3	1.8-11.2	na
	Field exposure	*Scrobicularia plana*	25	4.7 - 95 (mean: 27.2 ± 20.6)[c]	na
McCann pers. comm. (1999)	Field	oyster	5	0.05 - 0.45[a]	na
		mussel	5	0.04 - 0.30[a]	na
Meador et al. (1997)	Laboratory exposure	*Armandia brevis*, *Rhepoxynius abronius* and *Eohaustorius washingtonianus*	31	8.3 - 98	0.4 - 4.6
Meador pers. comm. (1999)	Laboratory exposure to field collected sediment	*Armandia brevis*	2	7.7	1.7
Stäb et al. (1996)	Field exposure (freshwater)	Chironomids	5	0.5 - 20	na
		Gammarids	4	0.62 - 8.64	na

NOTE: na - not applicable

[a] Range of potential BAF values calculated from the range of sediment and tissue concentrations.
[b] Mean BSAF calculated using mean lipid values from WSOU study.
[c] Mean calculated as the mean of 25 locations with 1–9 sites sampled per location.

3.4 *Armandia brevis* Data from near Todd Shipyard

Sediment bioaccumulation testing was conducted for a sample collected independently of the WSOU study in January 1999. The sample was collected near Todd Shipyard and *Armandia brevis* was the test organism (Meador pers. comm. 1999). The BAFs and BSAFs calculated from preliminary results for *A. brevis* were approximately an order of magnitude higher than the BAFs and BSAFs calculated for *N. caecoides* and *M. nasuta* during the WSOU sampling (Table 3-1). The *Armandia* testing was a static 14-day test with no sediment renewal.

3.5 Langston and Burt (1991)

TBT concentrations were measured in sediments and deposit-feeding clams, *Scrobicularia plana*, from 25 estuarine locations in England and Wales (Langston and Burt 1991). Laboratory bioaccumulation experiments were also conducted using the same species and TBT-spiked sediments. The calculated BAFs are presented in Table 3-1. Several of Langston and Burt's (1991) sites produced tissue residues in the low ppm range for sediment between 100 and 200 ppb TBT (250 to 500 ng TBT/g dry wt).

The authors compared the results of their field and laboratory studies to results obtained with other species of shellfish. They concluded that even among related species occupying similar habitats, there may be marked inter-specific differences in TBT bioaccumulation potential. These differences appear to be driven by differences in the abilities of the individual species to metabolize and excrete TBT, as well as differences in feeding strategies that affect the exposure of the organisms (Langston and Burt 1991).

A recent reinterpretation of the data presented by Langston and Burt (1991) has been presented by Salazar (1999a). Salazar (1999a) suggested that at higher TBT concentrations bioaccumulation is reduced. He proposed that the concentration at which reduced bioaccumulation is observed be used as an indicator of where sublethal effects begin to occur. With regard to the WSOU bioaccumulation results, Salazar (1999b) suggests that the relatively low tissue TBT concentrations observed after the WSOU bioaccumulation testing are the result of stress on the test organisms caused by the presence of elevated sediment TBT concentrations and by laboratory holding conditions. It is noted that for the WSOU testing, the survival of the test organisms was high, and the lipid content of the organisms exposed to test sediments was similar to controls.

3.6 FIELD STUDIES

Five studies in which field-collected sediment and tissue samples were analyzed for TBT also are summarized in Table 3-1 (Bryan and Gibbs 1991; King et al. 1989; Kure and Depledge 1994; Langston et al. 1987; Stäb et al. 1996). One study was conducted in a freshwater lake (Stäb et al. 1996) and the remaining studies were conducted in coastal marine waters. BSAF values could not be calculated due to the lack of sediment organic carbon data or tissue lipid data. The calculated BAF values ranged from 3 to 597.

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